Fractal analysis of the PDL-bone interface and implications for orthodontic tooth movement

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Background: The periodontal ligament (PDL) is a soft tissue interposed between the tooth and the alveolar bone. It is responsible for transmission of forces in vivo; this promotes bone remodeling. The purpose our study was to use fractal analysis to quantify the complex morphology of the PDL-bone interface. **Methods:** We used Scion Image (Scion Corp, Frederick, Md) and Benoit fractal analysis (Tru Soft International, St. Petersburg, Fla) programs to calculate the fractal dimension of the PDL-bone interface in rats via the box-counting method. Rats in the experimental groups received an initial force of 0.1N or 0.5N with customized springs for 6 hours. **Results:** Our studies showed an increase in normal fractal dimension at the root apices of the rats' maxillary molars. We also found evidence that the fractal dimension varies along the entire root length from the apex to the cementoenamel junction. **Conclusions:** Mechanical loading might lead to an increase in fractal dimension at the PDL-bone interface apart from mechanisms of bone cell directed remodeling. These changes in fractal dimension are proportional to loading and could provide a new parameter for force determination in orthodontic tooth movement. (Am J Orthod Dentofacial Orthop 2005; 127:655-61)

Mechanical forces exerted on the tooth and transmitted to the bone via the periodontal ligament (PDL) produce orthodontic tooth movement. The biological response of the periodontal tissues to loading as seen in mastication and orthodontic movement enables supporting bone to adapt to changes in its mechanical environment. It is therefore important to investigate biomechanical and structural reactions of the bone, the PDL, and the PDL-bone interface to external forces, to determine optimal force application in orthodontic tooth movement. The PDL-bone interface is an integral component of mechanotransduction in normal occlusion and orthodontic movement. Mechanical loading causes structural changes at the PDL-bone interface that have not been quantified because of its irregular morphology. Few investigations have studied this interface because its complexity does not allow for numerical assessment by Euclidean geometrical principles. Fractal analysis can, therefore, be used to quantify this interface.

Fractal geometry of an object is a measure of its self-similarity. It provides methods for characterizing complexity and thus quantifying morphologies that are generally considered irregular. "Fractal" is a concept introduced by Mandelbrot[1] to designate objects with "fractional" geometric dimensions. The fractal dimension of an object is a measure of its space-filling properties; the more space the object occupies, the higher the fractal dimension.[1] A fractal is a set for which the Hausdorff-Bescovich dimension strictly exceeds the topological or Euclidean dimension. This Hausdorff-Bescovich dimension is the fractal dimension of an object. Generally, the higher the fractal dimension, the more complex the object.[2] Fractals have been used to describe objects in nature that do not strictly follow Euclidean geometrical principles—eg, clouds, coastlines, and mountains.

Recent studies have shown that irregularity of junctions between tissues can be of significant diagnostic value. For example, irregularity of the junction between normal tissue and a tumor was determined to be the most significant diagnostic factor when assessing a lesion for malignancy.[3] It has been shown that the probability that a lesion is malignant increases with greater border irregularity and consequently increased fractal dimension.[4] In oral-floor lesions, irregularity of the epithelial connective tissue interface is used as a diagnostic feature, and higher irregularity correlates with locally invasive areas.[5]

Response of the PDL-bone interface to mechanical deformation is important in our understanding of changes induced by orthodontic tooth movement. In this study, we examined fractal dimensional change at

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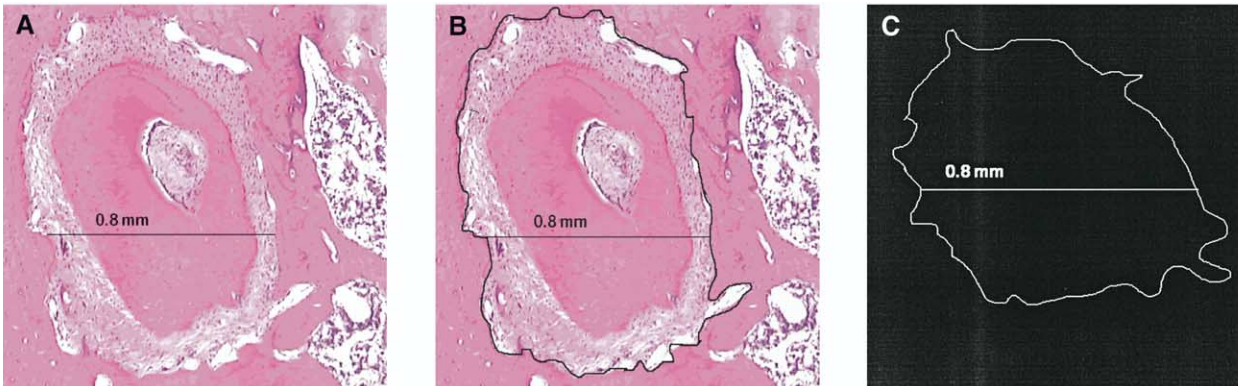

Fig 1. A, Hematoxylin-eosin staining, section of mesiolingual root of maxillary first molar. **B**, hematoxylin-eosin staining, with PDL-bone interface outlined; **C**, Outline converted to black and white to estimate fractal dimension.

the PDL-bone interface at the apex of the maxillary first and second rat molars in response to mechanical loading and its implications in orthodontic tooth movement. We also examined the variation in fractal dimension from the root apex to the cementoenamel junction in normal and experimental (force induced) maxillary first molar roots of rats. We hypothesized that force application would cause in increase in complexity of the PDL-bone interface and increase the fractal dimension. Our studies suggest that heavy tipping forces are responsible for PDL and bone remodeling, and this is manifested as increased fractal dimension at the PDL-bone interface. We believe that a physical factor and not cellular response (increased osteoblastic or osteoclastic activity) is responsible for the observed change in fractal dimension.

MATERIAL AND METHODS

Tooth movement

Twenty-four Sprague-Dawley retired female breeder rats (Harlan, Indianapolis, Ind) were used in this study. Each rat weighed between 250 and 300 g. They were maintained on a pellet rat diet and water ad libitum. The animal protocol was reviewed and approved by the Committee for Care and Use of Laboratory Animals at Medical College of Georgia. The animals were divided into 3 groups, 2 experimental and 1 control, of 8 rats each.

The experimental groups received an initial force of 0.1 N or 0.5 N with customized springs.[6] The rats were anesthetized, and springs were inserted into small circular depressions made with a dental handpiece on the lingual surface of the first molars near the mesiolingual cusp. The force was delivered from the lingual to the buccal direction, for a 6-hour period. The rats were killed immediately after the removal of the spring. The control group followed the same protocol, but no spring was placed across the maxilla.

Preparation of sections

The springs were removed under deep anesthesia, and the rats were perfused transcardially with 40 mL of 10% formalin solution until death. The maxilla was isolated by using a low-speed saw (Isomet Buehler Ltd, Lake Buff, Ill) under constant water irrigation. All soft tissue was removed from bone. The sections were processed through graded series of alcohol and xylene before embedding in paraffin.[6]

Serial sections 5 μm thick were cut in the occlusal plane through the molar roots from the mesial of the first maxillary molar to the distal of the third maxillary molar. Samples obtained from the 0.5-N application and the control group were sectioned from the apex to the furcation area. The samples from the 0.1-N force application were sectioned from the lowest one fifth of the root apex and compared with corresponding sections obtained from the control and 0.5-N force samples.

For the regional analysis, 5-μm sections were cut sequentially from the apical third (0-250μm), the middle third (250-500 μm), and the cervical third (500-750 μm) regions of the roots. Representative sections from every 50 μm were chosen for analysis. The mesiolingual and distobuccal roots from the control group and the 0.5-N force group were used in this study.

Histology and immunohistochemistry

The PDL-bone interface was localized by routine hematoxylin-eosin staining (Fig 1, *A*). The osteoblasts and the osteoclasts along the PDL-bone interface were localized by the modified avidin-biotin peroxidase technique[7,8] by using antibodies against osteocalcin (Biodesign Int,